Evaluation of antioxidant and breast cancer cytotoxicity of *Beta vulgaris* and *Daucus carota*methanol extracts

ABSTRACT:

Breast cancer is the world's most frequent malignancy among women. Many dietary antioxidants have been shown to help prevent oxidative stress, which has been linked to a variety of diseases, including cancer. Many chronic diseases, such as cancer, can be avoided by eating fruits and vegetables on a regular and balanced basis. The current study's goal is to assess the antioxidant and cytotoxic capabilities of methanolic extracts of *Beta vulgaris* and *Daucus carota* against MCF-7 human breast cancer cell lines. Therefore, the aim of the present study was to determine the phytochemical components, antioxidant capacity, total phenolic & flavonoid contents in the methanolic extracts of ***Beta vulgaris*and *Daucus carota*.** Further, MTT assay against breast cancer cell line (MCF-7) was performed for the evaluation of cytotoxic activity.The majority of secondary metabolites were found in both the aqueous and methanolic extracts of *Beta vulgaris* and *Daucus carota*. *Beta vulgaris* and *Daucus carota* have different total phenolic and flavonoid contents. TLC results showed many spots having different Rf values. Moreover, we observe comparable cytotoxic activity in both the extracts against MCF-7 cell lines. Our results reveal that the methanolic extracts of *Beta vulgaris* and *Daucus carota* has effective phytochemical constituents, antioxidant and anticancer activity. The total phenolic and flavonoid contents vary between *Beta vulgaris* and *Daucus carota.* Further studies are needed to evaluate the chemopreventive potentials of the *Beta vulgaris* and *Daucus carota* extract when used alone or in combination with doxorubicin to mitigate the toxic side-effects of the latter.

KEYWORDS: Antioxidant activity, cytotoxic potential, Breast Cancer, *Beta vulgaris, Daucus carota*

Running Title: Antioxidant and cytotoxic activity of beet root and carrot

1. INTRODUCTION:

Scientific knowledge regarding the antioxidant abilities of many plants is currently insufficient, particularly those less commonly used in food and medicine.Many of today's ailments are now known to be caused by "oxidative stress," which is produced by an imbalance in the generation and neutralization of prooxidants. When the free radicals are produced in excess, they interact with the body's numerous bio molecules and destroy cells [1][2]. Protein and DNA damage, as well as lipid peroxidation, which cause oxidative stress, are caused by free radicals, which seek stability by electron partnering with biological macromolecules such as proteins, lipids, and DNA in healthy human cells. These changes have been associated to cancer, atherosclerosis, cardiovascular disease, aging, and inflammatory disorders [2,3]. Oxidation and free radicals may play a role in carcinogenesis at multiple tumor sites because all cells are vulnerable to oxidative stress. According to data from numerous research, medicinal plants have a greater attention of natural antioxidants than typical dietary plants, including phenolics, flavonoids, and tannins [4].The majority of the antioxidants in our diets are polyphenols. One-third and two-thirds of polyphenols, respectively, come from flavanols (catechins plus proanthocyanidins), anthocyanins, and their oxidation products. Polyphenols are reducing agents that may protect the body's tissues from oxidative stress and associated pathologies like cancer, chronic heart disease, vascular diseases, and inflammation [5].

There are no metaphors horrifying enough to convey cancer, despite the fact that people use it as a metaphor for the worst aspects of life [6]. Cancer is one of the deadliest diseases, with over 100 different types arising from molecular changes within cells. It is the third leading cause of death worldwide, after cardiovascular and infectious disorders [7]. According to estimates, cancer kills 12.5% of the population (WHO, 2004). The most frequent cancer in women and the primary cause of cancer-related deaths worldwide is breast cancer[8],with an estimated 2.1 million new cases reported in 2018. It is the top cause of death in more than 100 nations [9], accounting for one out of every four cases of cancer in women. Incidence varies by metro and city, with Delhi having the highest rate, followed by Chennai, Bangalore, and Thirpuram District [10]. In addition, over half of all breast cancers (43.7%) are diagnosed at an advanced stage [11]. Despite major advances in breast cancer treatment, invasion and/or metastasis continues to be one of the top causes of death. Resistance to drugs by cancer cells is a major stumbling point in the treatment of cancer. This syndrome affects a variety of cancer patients, particularly those with blood cancers and solid tumors in the lungs, breast, ovaries, lower gastrointestinal system and other organs [12].

Cancer can be a cause of death due to a lack of effective treatments, the high cost of chemotherapeutic medications, and the side effects of anticancer drugs. As a result, researchers are still looking for effective naturally occurring anti-carcinogens that can prevent, slow, or reverse the progression of cancer. In the treatment of cancer, medicinal plants play a unique role. Plant-derived chemicals are thought to make up more than half of all anticancer medicines in one form or another [13,14]. Furthermore, fruits and vegetables are the most important foods for maintaining excellent health and meeting nutritional requirements [15]. Fruits and vegetables play a significant part in the diet because they supply critical minerals, vitamins, and other nutrients [16]. Many chronic diseases, including cancer, stroke, and heart disease, can be prevented by eating fruits and vegetables on a regular basis and in a balanced way [17]. Beet root (*Beta vulgaris*) is a prospective plant utilised in cardiovascular disorders because of its anticancer, carminative, emmenagogue, hemostatic, and renal protective characteristics [18]. Beet root is well-known for its antioxidant properties [19]. Moreover, it has gained popularity in recent years as a natural energy booster for sports person [20,21]. Hippocrates, the Father of Medicine, recommended beet root leaves for faster wound healing [22]. *Beta vulgaris* extracts (root) have been shown to have antihypertensive, hypoglycemic, antioxidant [23], anti-inflammatory, and hepatoprotective properties [22,24,25,26]. *Beta vulgaris* has received a lot of attention as a healthy functional food. Although scientific interest in beet root has only recently grown, reports of its usage as a natural remedy extend back to the Roman era [23].

Carrot (*Daucus carota*) is a highly nutritious root vegetable that is widely used for juice production [27,28]. It is a good source of β-carotene, vitamins and minerals. Many countries have increased their usage of carrot juicen[29]. Fiber, carotenoids, vitamins C and E, and phenolic substances are all abundant in carrots. Phenolic molecules with hydroxyl groups on the aromatic ring are found in all plant sections. These are either shikimate- or phenylpropanoid-derived secondary plant metabolites [30]. Reduced oxidative DNA damage [31] and enhanced levels of plasma antioxidants [32] are two of the physiological benefits of carrot juice consumption. Because of their high dietary content and typically good storage properties, carrots play a vital role in the nutrition of Western industrialised nations [33]. Carrots were placed tenth in terms of nutritional value and seventh in terms of contribution to nutrition among 38 other fruits and vegetables. Therefore, keeping in view of the above beneficial effects of *Beta vulgaris* and *Daucus carota*; the antioxidant potential and anti-cancerous properties were evaluated against breast cancer cell lines. Furthermore, total phenolic & flavonoid contents were determined and compared.

2. MATERIALS AND METHODS:

2.1.Materials:

The plant materials were purchased from the local market of Jhansi district of Uttar Pradesh in the month of January 2017. *Beta vulgaris* and *Daucus carota* were carefully cleaned in tap water, followed by de-ionized water, and allowed to dry at room temperature in the dark. To prevent contamination, they were frequently monitored. It was finally crushed with the help of grinder and the sample was stored in airtight bottles for further study.

2.2.Chemical reagents:

MTT was purchased from Sigma-Aldrich Co. (St Louis, MO, USA). All other chemicals and reagents used were of AR grade and were obtained from Himedia.

2.3.Extraction Procedure:

Aqueous and methanolic extraction was the two procedures used for extraction.

2.3.a.Aqueous Extract:

Aqueous extraction was performed as described elsewhere [34]. Different concentrations of dry powder of *Daucus carota* stem i.e. in conical flasks with an equal volume (100 ml) of deionized water, 5 gm and 10 gm were taken. For one hour at 90°C, both flasks were placed in the water bath. Flasks were filtered using filter paper and stored at 4°C after being allowed at room temperature for 1 hour to cool.

2.3.b. *Methanolic Extract:*

Methanolic extraction was performed as described elsewhere [35].80% methanol was used for extraction using the Soxhlet apparatus. The Soxhlet apparatus was filled with plant material and solvent, then run at 60°C until it became colourless with constant water flows to cool the condenser. Finally, the extract was collected and kept at 4°C in sealed bottles.

2.4.Phytochemical Analysis:

The phytochemicals were substances found in plants that naturally occur and may have an impact on one's health [36].As mentioned previously [37], phytochemical analysis was carried out.More detail procedures are mentioned elsewhere [38].

2.5.Thin layer chromatography:

Using analytical plates coated in silica gel-G of a 0.2 mm thickness, TLC was carried out on the methanoloic extracts. The following solvent was employed [39]: butanol, acetic acid, and water (4:1:1). The capillary process allows this mixture to migrate onto the silica-coated plates. After being heated for 20–25 minutes, the fully developed coated plate was air dried. The spots were found using a freshly made 0.2% ninhydrin solution.

The movement of the spots was expressed by its retention factor (Rf).

$$R f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

2.6.Antioxidant activity:

Using the phosphomolybdenum reduction test technique[40], the total antioxidant activity of *Beta vulgaris* and *Daucus carota* was determined. The extract was mixed with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 M sodium phosphate, and 4 M ammonium molybdate), 0.1 mL of various extract concentrations, and incubated at 95°C for 90 minutes. A typical blank solution had the appropriate volume of the same solvent as the samples/standard along with the same volume of methanol in place of the extract. The calibration curve was made using ascorbic acid concentrations (g/ml) in methanol as a standard. Using a spectrophotometer, the reaction mixture's absorbance was determined at 695 nm.

2.7.Total Phenolic Content (TPC) Estimation:

Using the Folin-Ciocalteu method, the total phenolic content was determined[41]. As a reference, gallic acid was used. The Folin-Ciocalteu reagent was mixed with 100 µl of various dilutions, 500 µl of water, and left to stand for 6 minutes. Following that, 500 ml of distilled water and 1 ml of sodium carbonate at a concentration of 7% were added to the reaction mixture. After 90 minutes, the absorbance at 760 nm was measured. Gallic acid equivalents (mg GAE/g) were used to calculate the total phenolic content. The experiments were all carried out in triplicate.

2.8.Total Flavonoid Content (TFC) Estimation:

Aluminum chloride compound formation was used to assess the extracts' flavonoid concentration[41]. In order to calculate the flavonoid content's quercetin equivalent, quercetin was employed as the reference. 500 µl of distilled water were mixed with 100 µl of quercetin and 100 µl of 5% sodium nitrate, and the mixture was let to stand for six minutes. Following the addition of 150 µl of 10% aluminium chloride solution, 200 µl of a 1M NaOH solution was added successively after 5 minutes of standing time. On a UV spectrophotometer, the reaction mixture's absorbance was measured at 510 nm. Quercetin equivalents (mgQE/g) were used to measure the overall flavonoid content. All the procedures were performed in triplicate.

2.9.Cell culture and MTT assay for in vitro anticancer study:

The human breast cancer cell lines MCF-7 was procured from American Type Culture collection (ATCC), USA and cultured in Dulbecco's modified eagle medium low glucose (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) at 37^0C in 5% CO_2. The MCF-7 cancer cells in exponential growth phase were seeded and incubated with different concentrations of the beet root, carrot extract and doxorubicin. The viability of the cells was assessed after 72 hrs at 37^0C in 5% CO_2. Each well received 20 µl of MTT (2mg/ml) in PBS and was incubated at 37^0C for 3 hrs. After 3 hrs, MTT medium was withdrawn, the produced formazan crystals were dissolved with 100 µl of DMSO, and the absorbance was measured at 540 nm with microplate reader [42]. The cell viability was calculated as follows:

$$\% \text{ Cell viability} = 1 - \frac{T}{C} \times 100$$

2.10. Statistic analysis:

The data is presented as the mean SD of three separate experimental tests, and each test was done three times. One-way analysis of variance was used to analyse the data statistically (ANOVA). Significant differences between the means of parameters were determined ($p < 0.05$).

3. RESULTS:

3.1 ***Phytoconstiteunts:***

The presence or absence of phytoconstiteunts depends on the tests used for the qualitative detection of secondary metabolites. Moreover, variuos secondary metabolites are present in the aqueous and methanolic extracts of *Beta vulgaris* and *Daucus carota* (Table-1).

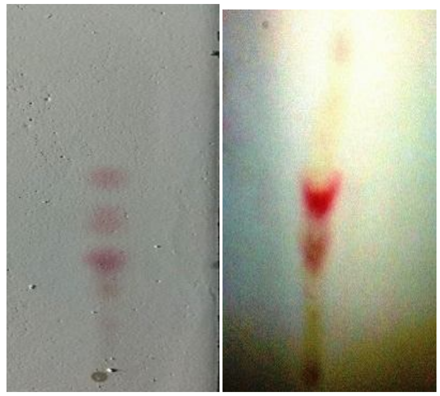
3.2. ***Thin layer chromatography:***

TLC of the methanolic extracts of *Beta vulgaris* shows positive results and total five spots were present having Rf values 0.18, 0.26, 0.35, 0.46, and 0.61. Whereas, *Daucus carota* alsoshows 5 spots having Rf values0.23, 0.35, 0.47, 0.60, and 0.67 (Fig. 1).

Table-1: Phytochemicals analysis of methanolic extracts of *Beta vulgaris* and *Daucus carota*.

S. NO	PHYTOCHEMICAL TESTS	*Beta vulgaris*	*Daucus carota*		
			METHANOLIC EXTRACT	**AQUEOUS EXTRACT**	
				5gm/100ml	**10gm/100ml**
1.	**TESTS FOR ALKALOIDS**				
	(A)Mayer's test	+ve	+ve	-ve	-ve
	(B)Wgner's test	+ve	+ve	+ve	+ve
	(C)Hager's test	+ve	+ve	+ve	+ve
2.	**TEST FOR CARBOHYDRATE**				
	(A)Molisch test	-ve	+ve	+ve	+ve
	(B)Barfoed's test	+ve	-ve	-ve	-ve
3.	**TEST FOR REDUCING SUGAR**				
	(A)Fehling's test	+ve	+ve	+ve	+ve
	(B)Benedict test	+ve	+ve	+ve	+ve
4.	**TEST FOR FLAVONOIDS**				
	(A)Alkaline reagent	+ve	+ve	+ve	+ve
	(B)Lead acetate	+ve	+ve	+ve	+ve
	(C) Ammonia test	+ve	-ve	-ve	-ve
5.	**TEST FOR GLYCOSIDES**				
	(A)Borntrager's test	-ve	-ve	-ve	-ve
	(B)Legal's test	-ve	-ve	-ve	-ve
	(C)10% NaOH test	-ve	-ve	-ve	-ve
6.	**TEST FOR CARDIAC TEST**				
	(A)Keller killani test	+ve	-ve	-ve	-ve
7.	**TEST FOR TANNIN AND PHENOLIC COMPOUND**				
	(A)Ferric chloride 5%	+ve	-ve	-ve	-ve
	(B)Lead acetate	+ve	+ve	+ve	+ve
	(C)Dilute iodine	+ve	+ve	-ve	-ve
	(D)Ferric chloride	+ve	-ve	-ve	-ve
	(E)Hydrolysable tannin	-ve	-ve	-ve	-ve
8.	**TEST FOR SAPONIN**				
	(A)Froth test	+ve	+ve	+ve	+ve
9.	**TEST FOR AMINO ACID AND PROTEIN**				
	(A)Ninhydrin test	+ve	+ve	+ve	+ve
	(B)Biuret test	-ve	+ve	-ve	-ve
10	**TEST FOR**	-ve	-ve	-ve	-ve

.	**TRITERPENOID**				
11.	**TEST FOR STEROID**	-ve	-ve	-ve	-ve



A B

Fig.-1: TLC Plate showing spots having different Rf Values of Methanolic extract of *Beta vulgaris* and *Daucus carota*

3.3. Antioxidant capacity, total phenolic & flavonoid contents:

Beta vulgaris and *Daucus carota* were evaluated for their overall antioxidant potential, and the results revealed dose-dependent activities. In comparison to *Beta vulgaris* extracts, *Daucus carota* extracts exhibit stronger antioxidant potential. We were interested in examining the overall phenolic and flavonoid levels because we saw antioxidant activity in the *Beta vulgaris* and *Daucus carota* extracts.There are not many differences in terms of total phenolic contents in both the study materials and the mean values are 116.85 mgGAE/g and 109.75 mgGAE/g for*Daucus carota* and *Beta vulgaris,* respectively. While the totalflavonoid contents varies between *Daucus carota* and *Beta vulgaris* and are 803 (mgQE/g) and 297(mgQE/g),respectively (Fig. 2, Table 2).

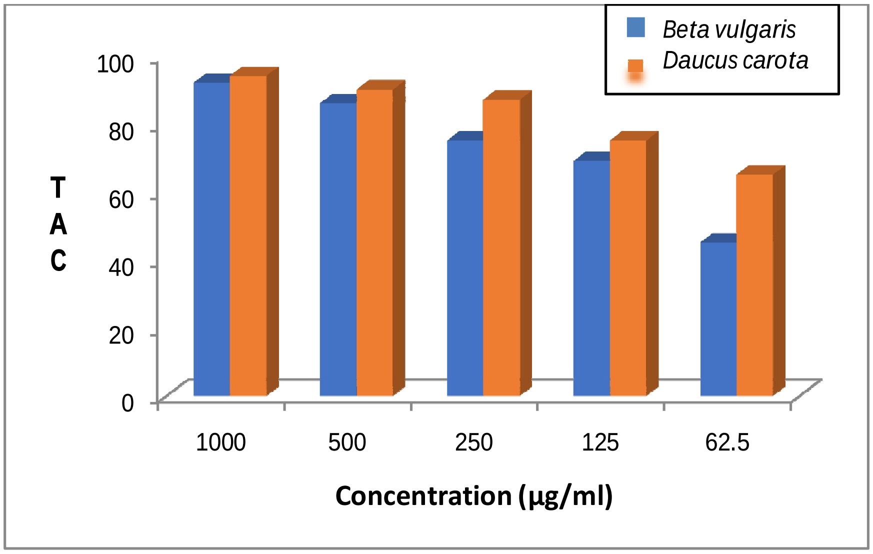


Fig-2: Total antioxidant capacity (TAC) of methanolic extract of *Beta vulgaris* and *Daucus carota*

Table:2- Total Flavonoid & Phenolic Content ofmethanolic extract of *Beta vulgaris* and *Daucus carota*

Total Flavonoid Content (mgQE/g)			**Total Phenolic Content (mgGAE/g)**		
Conc. (µg/ml)	*Beta vulgaris*	*Daucus carota*	**Conc. (µg/ml)**	*Beta vulgaris*	*Daucus carota*
1000	299	157	150	79.59	69.32
500	452	296	120	79.16	78.33
250	816	392	90	95.55	91.11
125	992	368	60	113.32	106.66
62.5	1456	272	30	216.66	203.33
Mean values	**803**	**297**		**116.85**	**109.75**

3.4.Cytotoxic Activity:

We tested methanolic extracts of *Daucus carota* and *Beta vulgaris* to verify the possible anti-proliferative effect as a first step toward the development of novel putative anticancer agents. Cell proliferation assays were performed to test the possible cytotoxicity of *Daucus carota* and *Beta vulgaris* extracts and doxorubicin (reference control). At doxorubicin concentrations of 25, 50, and 100 µg/ml, at which growth is inhibited by approximately 50, 90, and 100%, respectively. Extracts showed dose dependent activity in MCF-7 cell lines (Fig. 3). Cell viabilities were between 35 and 40 % at a concentration of 400 µg/ml.

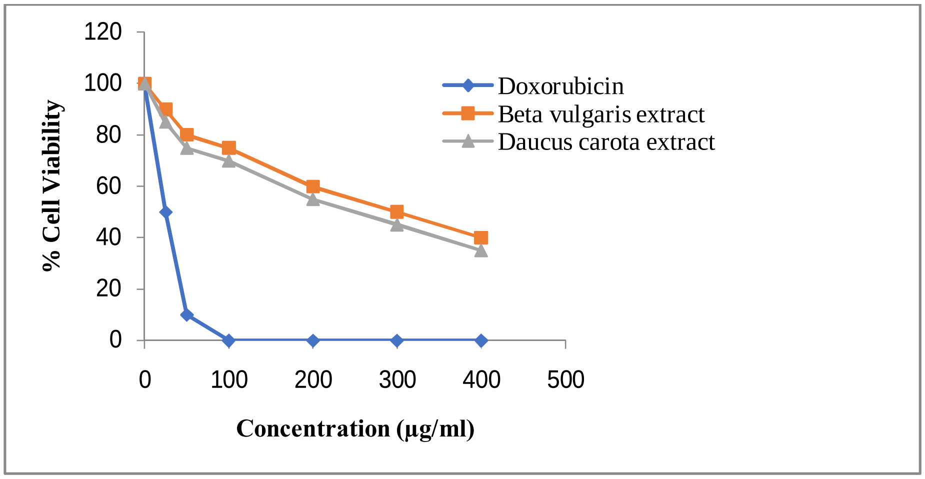


Fig- 3: Cytotoxic effects of the *Beta vulgaris*,*Daucus carota extract* andDoxorubicin in the human breast cancer cell lines MCF-7 after 72 hours of exposure. The MCF-7 cells were incubated with various concentrations of extracts/reference drug and the viability of cells was assessed after 72 hours using MTT assay. Experiments were performed in triplicate and expressed as the mean ± standard deviation. P <0.05 showed significant differences.

4. DISCUSSION:

Many living things depend on oxidation to produce the energy needed to power their metabolic functions [43]. Currently, one of the most prevalent diseases is oxidative stress, which results in an imbalance between the production and removal of reactive free radicals [44]. The antioxidant properties of fruits and vegetables from south India have been investigated widely on the individual basis with different analytical methods, and it is difficult to compare and correlate. Therefore, the present study was aimed to measure and compare the total antioxidants and cytotoxic potential of the selected and widely consumed fruits and vegetables from Bundelkhand region of India. The region is characterized as hot semi arid eco-region along with growing period of 90-150 days. The annual rainfall ranges from 838.6-1251 mm over the region which is often erratic. *Daucus carota* and *Beta vulgaris* extract showed various secondary metabolites. Both the extracts showed 5 spots having different Rf values. The total phenolic and flavonoid contents are comparable between *Beta vulgaris* and *Daucus carota*. Our results show dose dependent antioxidant and cytotoxic activity.

Doxorubicin (adriamycin; an anticancer antibiotic) is currently being utilised to treat a number of malignancies [45]. Sarcomas, lymphomas, mesothelioma, multiple myeloma, neuroblastoma, and certain kinds of leukaemia are among them. Doxorubicin treatment, on the other hand, is linked to a number of significant side effects [46,47,48,49]. Nausea, vomiting, and cardiac rhythms are all documented as acute adverse effects of doxorubicin. It can also result in neutropenia and complete alopecia. When the total dose of doxorubicin reaches 550 mg/ml, the chances of developing cardiac adverse effects such as congestive heart failure, dilated cardiomyopathy, and even mortality skyrocket. A dose-dependent decrease in mitochondrial oxidative phosphorylation is a hallmark of doxorubicin cardiotoxicity. Doxorubicin (adriamycin) has received the nicknames "red devil" and "red death" because of these side effects and its red colour, which causes urine, tears, and sweat to turn pinkish red [48,49].

The effects of the red beetroot, carrot extract and doxorubicin on the percent viability of cancerous MCF-7 cells are shown in Figs.-3. Doxorubicin was chosen for comparison because they both have an intense red colour and a planar aromatic chromophore attached to a six-membered sugar molecule in their chemical structure. The cytotoxic effects of beetroot, carrot extract, and doxorubicin were dose dependant. The cytotoxicity of the beetroot and carrot extracts in the MCF-7 cancer cell lines was significantly decreased at all concentration levels examined when compared to doxorubicin.

The colourants found in red beetroot are known as betalains, and they are divided into two groups: red betacyanins and yellow betaxanthines [50,51]. Since they were discovered as natural antioxidants with free radical scavenging properties and possible health benefits [50,51,52,53], food and nutritional supplement makers have been interested in betalains. Beetroot extract is ideal for colouring frozen, dry, and short-shelf-life items like ice cream, yoghurt, powdered beverage mixes, and fruit and cream fillings in confectionery. There are several chemical constituents in red beetroot extract [54,50,51,52], and the chemical structure and configuration of betanin are very comparable. The presence of a planar aromatic chromophore and a six-membered sugar molecule, both of which have been hypothesised as potential active sites for doxorubicin and its analogues intercalation with DNA in cancer cells [55,56], are particularly noteworthy. This shows that betanin, like doxorubicin and other anthracycline chemotherapy medicines may play a key role in the cytotoxic effect of red beetroot extract. Another mechanism of chemoprevention by beetroot betacyanins has recently been proposed [54], which is based on antagonising lymphocyte infiltration and reducing inflammation during the development of esophageal tumours in rats. Because betacyanins are powerful antioxidants [52,57], it's not surprising that they can help fight against inflammation. Thus, one of the mechanisms of cancer chemoprevention by betanin, the primary betacyanin ingredient with very strong free radical scavenging activity [58], could be the lowering of inflammation.

Betaine (trimethylglycine, occurring at a concentration of up to 1.5% dry weight) is one of the additional ingredients of beetroot extract that may serve as a cytotoxic agent by methylating DNA in cancer cells[45]. However, based on large population studies [59,60,61], such as the 1984–2004 Nurse's Health Study [60], there is still no consensus on an association between dietary consumption of betaine (together with choline) and cancer mortality rate. Betanin also suppresses the activity of DNA methyltransferase in human breast cancer MCF-7 cells [62], but the relevance of this has yet to be determined.

Carrots (*Daucus carota* L.) are the most common root vegetable, farmed all over the world and high in natural phytochemicals. Carrot root is popular due to its high levels of carotenoids, anthocyanins, and nutritional fibre. Carrots are the primary source of carotenoids, and prior research has shown that they may reduce cancer risk and play a key part in cancer prevention diets [63,64,65]. Carrots have long been used in Lebanon to cure gastric ulcers, diabetes, muscle discomfort, and cancer [66]. Carrots have been shown to have antibacterial, antifungal, diuretic, antilithic, anticancer, anti-inflammatory, and anti-oxidant properties [66,67,68,69,70]

Liu et al. used silica gel, Sephadex LH-20, and preparative HPLC to purify methanol extracts of carrot root in order to isolate a chemical that targets mammosphere production. They also discovered 6-methoxymellein, a chemical that inhibits breast cancer cell proliferation and migration, reduces mammosphere formation, lowers the fraction of $CD44^+/CD24^-$ cells in breast cancer cells, and lowers the expression of stemness-associated proteins[71]. c-Myc, Sox-2, and Oct4 are three genes that have been linked to cancer. The nuclear localization of nuclear factor-κB (NF-κB) subunits p65 and p50 is reduced by 6-methoxymellein. As a result, 6-methoxymellein reduces IL-6 and IL-8 mRNA transcription and secretion. They suggested that 6-methoxymellein may be an anticancer agent that inhibits breast cancer stem cells via NF-κB/IL-6 and IL-8 regulation. Sevimli-Gur et al. investigated the effects of black carrot extracts on a number of human cancer cell lines, including VERO (African green monkey kidney) normal cell line, HT-29 (human colon adenocarcinoma), MCF-7 SK-BR-3 and MDA-MB-231(human breast adenocarcinomas), PC-3 (human prostate adenocarcinoma), and Neuro 2A (Musmusculus neuroblastoma). According to their report, Neuro-2A cell lines exhibited the highest level of cytotoxic activity[72]. Thus, our finding is supported by previous studies. Moreover, to reduce the toxic side-effects of doxorubicin, more research is needed to examine the chemopreventive potentials of *Beta vulgaris* and *Daucus carota* extracts when taken alone or in conjunction with it.

5. CONCLUSIONS

In addition to providing the phytochemical profile of the methanolic extract of *Beta vulgaris*and *Daucus carota*, this work also demonstrates the anti-proliferative potential of the two extracts against breast cancer cell line. The phytochemicals included in the extracts are responsible for the anti-proliferative action of these extracts. This work establishes a crucial foundation for future research on the use of *Beta vulgaris*and *Daucus carota*herbal medicine as an alternative therapy for the management and treatment of cancer. Further, this study suggests more research to isolate the bioactive ingredients from the extracts and elucidate how they function.

CONSENT AND ETHICAL APPROVAL

Not applicable

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to Bundelkhand University, Jhansi, Uttar Pradesh, India, for providing support during the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Patel DS, Shah PB, Managoli NB. Evaluation of In-vitro Anti-oxidant and Free Radical Scavenging activities of Withania somnifera and Aloe vera. Asian J. Pharm. Tech. 2012;2(4):143-147.
2. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from Licanialicaniae flora. J Ethnopharmacol 2002;79(3):379–81.
3. Maxwell SR. Prospects for the use of antioxidant therapies. Drugs. 1995;49(3):345–361.
4. Ch. Madhu, J. Swapna, K. Neelima , Monic V. Shah. A Comparative Evaluation of the Antioxidant Activity of Some Medicinal Plants Popularly Used in India. Asian J. Res. Pharm. Sci. 2012;2(3):98-100.
5. Ghanshyam B. Jadhav, Ravindranath B. Saudagar. Free radical Scavenging and Antioxidant Activity of Punica granatum Linn. Asian J. Res. Pharm. Sci. 2014;4(2): 51-54
6. Lavanya, Nalini Jeyavantha Santha, Gowri Sethu. A Study to Assess the Effectiveness of Exercise on Cancer-Related Fatigue among Women with Breast Cancer Admitted in Erode Cancer Centre, Erode. Int. J. Adv. Nur. Management 2014;2(3):134-138.
7. Kelloff GJ. Perspectives on cancer chemoprevention research and drug development. Adv Cancer Res 2008;78:199–334.

8. Akshay R. Yadav, Shrinivas K. Mohite. Cancer- A Silent Killer: An Overview. Asian J. Pharm. Res. 2020;10(3):213-216.
9. Bray F, Ferlay J, Soerjomataram I, Siegel RL. Lindsey A Torre, Jemal A. Global cancer statistics GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
10. Hussain S, Singh A, Nazir SU, Tulsyan S, Khan A, Kumar R, Bashir N, Tanwar P, Mehrotra R. Cancer drug resistance: a fleet to conquer. J Cell Biochem. 2019;120(9): 14213-14225.
11. Soliman AS, Samad S, Banerjee M, Chamberlain RM, Robert M, Aziz Z. Brief Continuing Medical Education (CME) Module Raises Knowledge of Developing Country Physicians. Int Electron J Health Educ.2006;9:31–41.
12. Nazir SU, Kumar R, Rasool I, Bondhopadhyay B, Singh A, Tripathi R, Singh N, Khan A, Tanwar P, Agrawal U, Mehrotra R. Differential expression of Ets□1 in breast cancer among North Indian population. J Cell Biochem. 2019;120(9):14552-14561.
13. Babior BM. Phagocytes and oxidative stress. Am J Med. 2000;109(1): 33–44.
14. Nipun D, Vijay S, Jaykumar B, Kirti SP, Richard L. Antitumor Activity of Dendrophthoefalcata against Ehrlich Ascites Carcinoma in Swiss Albino Mice. Pharma Crops2011;2(1):1–7.
15. Wong P, Yusof S, Ghazali HM, Cheman B. Optimization of Hot Water Extraction of Roselle Juice using response Surface Methodology: A Comparative Study with other extraction methods. J. Sci. Food Agric. 2003;83(12):1273–1278.
16. Ragaert P, Verbeke W, Debevere F. Consumer Perception and Choice of Minimally Processed Vegetables and Packaged Fruits. Food Qual Prefer. 2004;15(3):259–270.
17. Van Duyn MS, Pivonka E. Overview of the health benefit of fruits and vegetable consumption for dietetics professional: selected literature. J. Am. Diet. Assoc 2000; 100(12):1511-1516.
18. Vali L, Stefanovits-Banyai E, Szentmihalyi K, Febel H, Sardi E, Lugasi A, KocsisI, Blazovics A. Liver protecting effects of table beet (Beta vulgaris var.rubra) during ischemia-reperfusion. Nutrition. 2007;23(2):172–178.
19. Winkler C, Wirleitner B, Schroecksnadel K, Schennach H, Fuchs D. In vitro effects of beet root juice on stimulated and unstimulated peripheral blood mononuclear cells. Am J Biochem Biotechnol 2005;1(4):180-185.
20. Ormsbee MJ, Bach CW, Baur DA. Pre-exercise nutrition: the role of macronutrients, modified starches and supplements on metabolism and endurance performance. Nutrients 2014; 6(5):1782–1808.
21. Ormsbee MJ, Lox J, Arciero PJ. Beetroot juice and exercise performance. Nutr Diet. Suppl. 2013;5:27-35.
22. Singh A, Garg VK, Sharma PK, Gupta S. Wound healing activity of ethanolic extract of Beta vulgaris. Pharmacology online. 2011;1:1031–1038.
23. Ninfali P, Angelino D. Nutritional and functional potential of Beta vulgaris cicla and rubra. Fitoterapia. 2013;89:188–199.

24. Chakole R, Zade S, Charde M. Antioxidant and anti-inflammatory activity of ethanolic extract of Beta vulgaris Linn. Roots. Int. J. Biomed. Adv. Res. 2011; 2(4):124–130.
25. Jain S, Garg VK, Sharma PK. Anti-inflammatory activity of aqueous extract of Beta vulgaris L. J. Basic Clin. Pharm. 2011;2(2):83.
26. Kujala TS, Loponen JM, Klika KD, Pihlaja K. Phenolics and betacyanins in red beetroot (Beta vulgaris) root: Distribution and effect of cold storage on the content of total phenolics and three individual compounds. J. Agric. Food Chem. 2000; 48(11):5338-5342.
27. Demir N, Acar J, Baheci KS. The use of commercial pectinase in fruit juice industry. Part 3: Immobilized pectinase for mash treatment. J. Food Eng. 2000;47(4):275-280.
28. Walde SG, Math RG, Chakkarvarthi A, Rao DG. Preservation of carrots by dehydration techniques—A Review. Indian Food Packer. 1992;46:37–42.
29. Schieber AM, Carle R. Simultaneous determination of carotenes tocopherol in ATBC drinks by high-performance liquid chromatography. J. Food Chem. 2001;76(3):357-362.
30. Muhammad Hamza Ashfaq, Amna Siddique, Sammia Shahid. Antioxidant Activity of Cinnamon zeylanicum: (A Review). Asian Journal of Pharmaceutical Research. 2021; 11(2):106-6.
31. Pool-Zobel B, Liegibel UM, Treptow-van LS, Rechkemmer G. Mechanisms by which vegetable consumption reduces genetic damage in humans Cancer. Epidemiol Biomarkers Prev. 1998;7(10):891-899**.**
32. Törrönen RM, Lehmusaho M, Häkkinen S, Hänninen O, Mykkänen H. Serum β-carotene response to supplementation with raw carrots, carrot juice or purified β-carotene in healthy non-smoking women. Nutr Res.1996;16(4):565-575.
33. Berger M, Küchler T, Maaben A, Busch-Stockfisch M, Steinhart H. Correlations of carotene with sensory attributes in carrots under different storage conditions. J. Food Chem. 2008;106(1):235–240.
34. Singh V, Kumar R. Study of Phytochemical Analysis and Antioxidant Activity of Allium sativumof Bundelkhand Region. Int. J. Life. Sci. Scienti. Res.2017;3(6):1451-1458.
35. Kumar R, Patwa R. Study of Phytochemical Constituents and Anti-oxidant Activity of *Spinaciaoleracea*L. of Bundelkhand Region. Int J Life Sci Scienti Res.2018;4(1):1599-1604.
36. Ermi Abriyani, Lia Fikayuniar. Screening Phytochemical, Antioxidant Activity and Vitamin C Assay from Bungo perak-perak (Begonia versicolar Irmsch) leaves. Asian J. Pharm. Res. 2020;10(3):183-187.
37. Sharma S, and Kumar R. Antioxidant activity, TLC and phytochemical analysis of ginger (*Zingiber officinale*L.) rhizome. Plant Archives Special Issue ICAAAS-2018; 18:210-221
38. Singh V, Kumar R. Study of Phytochemical Analysis and Antioxidant Activity of Allium sativum of Bundelkhand Region. Int. J. Life. Sci. Scienti. Res. 2017;3(6):1451-1458.

39. Kumar R, Kumar D, Patwa R. Evaluation of phytochemical constituents and antioxidant activity of *Chenopodium album* of Bundelkhand region. J. Pharm. Res 2018;12(1):81-86.
40. Kumar D, Sharma S, Kumar R. Free radical scavenging activity of the mixture of different medicinal plants of Bundelkhand region. J. Pharm. Res. 2020;10(2):1008–1019.
41. Kumar R, Sharma S, Devi L. Investigation of Total Phenolic, Flavonoid Contents and Antioxidant Activity from Extracts of *Azadirachta indica*of Bundelkhand Region. Int J Life Sci Scienti Res. 2018;4(4):1925-1933.
42. Dwivedi V, Shrivastava R, Hussain S, Ganguly C, Bharadwaj M. Cytotoxic Potential of Indian Spices (Extracts) Against Esophageal Squamous Carcinoma Cells. Asian Pac J Cancer Prev 2011;12(8):2069–2073.
43. Anitha Jebamalai Raj, Sudarsanam Dorairaj. Phytochemical Screening and In-vitro Antioxidant Activity of Cissus quadrangualris. Asian J. Research Chem. 2010;3(4): 876-878.
44. Messaoudi Abdeldjabbar, Dekmouche Messaouda, Rahmani Zhour, Bensaci Cheyma. Comparative Analysis of Total Phenolics, Flavonoid content and Antioxidant profile of date palm (Phoenix dactylifera L.) with different watering water from Oued Soufin Algeria; Asian J. Research Chem. 2020;13(1):28-32.
45. Lown JW. Anthracycline and anthraquinone anticancer agents: Current status and recent developments. Pharmacol. Ther.1993;60(2):185-214.
46. DOXIL Product Information Sheet, Ortho Biotech Products, L.P., Horsham, PA; 2007.
47. Bloch A. Fighting Cancer: 25 Most Asked Questions, R.A. Bloch Cancer Foundation, Kansas City, MO; 2007.
48. Groopman JE. How Doctors Think, Houghton Mifflin, Boston, Massachusetts, USA; 2007, p. 320.
49. Carvalho C, Santos RX, Cardoso S, Coorria S, Oliveira PJ, Santos MS, Moreira PI. Doxorubicin: The good, the bad and the ugly effect. Curr. Med. Chem.2009;16(25):3267-3285.
50. Azeredo HMC. Betalains: properties, sources, applications, and stability – a review. Int J Food Sci Technol. 2009;44(12):2365–2376.
51. Stintzing FC, Carle R. N-Heterocyclic pigments: Betalains. In: Food Colorants: Chemical and Functional Properties. Socaciu C, editor. Raton, FL: CRC Press, Boca; 2008 p. 87-93.
52. Escribano J, Pedreno MA, Garcinia-Camona F, Mufioz R. Characterization of the antiradical activity of betalains from Beta vulgaris L. roots. Phytochem Anal. 1998; 9(3):124–127.
53. Tesoriere L, Allegra M, Butera D, Livera MA. Absorption, excretion and distribution of dietary antioxidant betalains in LDLs: Potential health effects of betalains in humans. Ame J Clin Nutr. 2004;80(4):941–945.
54. Lechner JF, Wang LS, Rocha CM, Larue B, Henry C, McIntyre CM, Riedl KM, Schwartz SJ, Stoner GD. Drinking water with red beetroot food color antagonizes esophageal carcinogenesis in N-nitrosomethylbenzylamine-treated rats. J Med Food 2010;13(3):733–739.
55. Formari FA, Randolph JJ, Yalowich JC, Ritke MK, Gewirtz DA. Interference of doxorubicin with DNA unwinding in MCF-7 breast tumor cells. Mol Pharmacol. 1994;45(4):649–656.
56. Pigram WJ, Fuller W, Hamilton LD. Stereochemistry of interactions: interaction of daunomycin with DNA. Nat New Biol. 1972;235(53):17–19.

57. Kanner J, Harel S, Granit R. Betalains – a new class of dietary cationized antioxidants. J Agri Food Chem. 2001;49(11):5178–5185.
58. Gliszczynska-Swiglo A, Szymusiak H, Malinowska P. Betanin, the main pigment of red beetroot: molecular origin of its exceptionally high free radical-scavenging activity. Food Addit. Contam 2006;23(11):1079-1087.
59. Xu X, Gammon MD, Zeisel SH, Bradshaw PT, Wetmur JG, Teitelbaum SL, et al. High intakes of choline and betaine reduce breast cancer mortality in a population-based study. FASEB J. 2009;23(11):4022–4028.
60. Cho E, Holmes MD, Hankinson SE, Willett WC. Choline and betaine intake and risk of breast cancer in post-menopausal women. Br J Cancer. 2010;10(3):489–494.
61. Lee JE, Giovannucci E, Fuchs CS, Willett WC, Zeisel SH, Cho E. Choline and betaine intake and the risk of colorectal cancer in men. Cancer Epidemiol Bio-markers Prev. 2010;19(3):884–887.
62. Paluszczak J, Ktajka-Kuzniak V, Baer-Dubowska W. The effect of dietary polyphenols on the epigenetic regulation of gene expression in MCF7 breast cancer cells. Toxicol Lett. 2010;192(2):119–125.
63. Sharma KD, Karki S, Thakur NS, Attri S. Chemical composition, functional properties and processing of carrot-a review. J Food Sci Technol. 2012;49(1):22–32.
64. Zaini RG, Brandt K, Clench MR, Le Maitre CL. Effects of bioactive compounds from carrots (Daucus carota L.), polyacetylenes, beta-carotene and lutein on human lymphoid leukaemia cells. Anticancer Agents Med Chem. 2012;12(6):640–652.
65. Sevimli-Gur C, Cetin B, Akay S, Gulce-Iz S, Yesil-Celiktas O. Extracts from black carrot tissue culture aspotent anticancer agents. Plant Foods Hum. Nutr.2013; 68(3):293–298.
66. Daaboul HE, Daher CF, Bodman-Smith K, Taleb RI, Shebaby WN, Boulos J. Antitumor activity of beta-2-himachalen-6-ol in colon cancer is mediated through its inhibition of the pi3k and mapk pathways. Chem Biol Interact. 2017;275:162–170.
67. Staniszewska M, Kula J, Wieczorkiewicz M, Kusewicz D. Essential oils of wild and cultivated carrots—The chemical composition and antimicrobial activity. J Essent Oil Res. 2005;17(5):579–583.
68. Maxia A, Marongiu B, Piras A, Porcedda S, Tuveri E, Goncalves MJ, Cavaleiro C, Salgueiro L. Chemical characterization and biological activity of essential oils from Daucuscarota l. Subsp. Carota growing wild on the mediterranean coast and on the atlantic coast. Fitoterapia.2009;80(1):57–61.
69. Shebaby WN, El-Sibai M, Smith KB, Karam MC, Mroueh M, Daher CF. The antioxidant and anticancer effects of wild carrot oil extract. Phytother Res. 2013; 27(5):737–744.
70. Shebaby WN, Daher CF, El-Sibai M, Bodman-Smith K, Mansour A, Karam MC, Mroueh M. Antioxidant and hepatoprotective activities of the oil fractions from wild carrot (*Daucus carota* ssp. Carota). Pharm. Biol.2015;53(9):1285–1294.
71. Liu R, Choi HS, Kim SL, Kim JH, Yun BS, Lee DS. 6-Methoxymellein isolated from carrot *(Daucus carota* L.) targets breast cancer stem cells by regulating NF-κB signaling. Molecules. 2020;25(19):4374.
72. Sevimli-Gur C, Cetin B, Akay S, Gulce-Iz S, Yesil-Celiktas O. Extracts from black carrot tissue culture as potent anticancer agents. Plant Foods Hum Nutr. 2013; 68(3):293-298.